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Eyeing Up New Wnt Pathway Players

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The Wnt signaling pathway controls cell fate decisions during animal development and the self-renewal and repair of adult tissues. In this issue, Ye et al. (2009) and Junge et al. (2009) reveal how an unusual Wnt receptor-ligand complex associated with a spectrum of retinal diseases instructs vascular development in the retina.

It is somewhat perplexing that an apparently limitless diversity of biological events can be controlled by reiterative use of the same, relatively simple signaling pathway. This may partly be due to the fact that many of the key Wnt pathway components occur more than once in animal genomes, such that multiple parallel versions of the pathway can operate in the same animal. The mammalian genome encodes two to four versions of many pathway components; however most diversity occurs at the ligand-receptor level. For example, mammals have around 20 Wnt ligands and 10 Frizzled receptors, which in different combinations regulate a variety of different processes including cell fate decisions during development and tissue repair in the adult. In this issue of *Cell*, Ye et al. (2009) and Junge et al. (2009) report on a group of hereditary retinopathies, including Norrie disease, that are associated with mutations in Wnt ligands and their Frizzled receptors. These two studies investigate how the ligand Norrin (which is not related to Wnt ligand) activates the canonical Wnt signaling pathway via the Frizzled-4 receptor leading to activation of β-catenin and vascularization of the developing retina. They also identify a new player in the Norrin/β-catenin signaling pathway and shed light on the diversification of the Wnt signaling pathway at the ligand-receptor level.

The retinal vasculature is laid down in a simple and stereotyped architecture during development. The major arteries and veins reside on the inner surface of the retina and project radially outward from the optic disc. Smaller branches penetrate the retina and drain two capillary beds located on either side of a central layer of neurons (the inner nuclear layer). Hypovascularization of the retina is a shared hallmark of a cluster of hereditary retinopathies including Norrie disease, familial exudative vitreoretinopathy, and osteoporosis pseudoglioma syndrome. Loss-of-function mutations responsible for these ophthalmic diseases are known to occur in the genes encoding the cysteine-knot protein Norrin, the Wnt receptor Frizzled-4, and its coreceptor low-density lipoprotein 5 (Lrp5) (Berger and Ropers, 2001; Warden et al., 2007).

Although structurally unrelated to Wnt proteins, Norrin is a direct ligand for the Frizzled-4/Lrp5 complex, a component of the canonical Wnt signaling pathway, as evidenced by the following observations. First, the retinal hypovascularization phenotypes of the respective mouse knockout models resemble each other. Second, Norrin binds with high affinity and specificity to Frizzled-4, and coexpression of Norrin, Frizzled-4, and Lrp5 potently activates Wnt signaling (Kato et al., 2002; Xu et al., 2004; Richter et al., 1998; Luhmann et al., 2005; Xia et al., 2008). Junge et al. (2009) and Ye et al. (2009) now provide insight into how Norrin activates canonical Wnt/β-catenin signaling through Frizzled-4 and Lrp5 to control vascularization of the retina during development.

Although Norrin/β-catenin signaling is known to be required for retinal vascular development, it was not clear whether this pathway was activated in neurons, glia, or endothelial cells in the retina. Combining mouse genetic and cell culture approaches, Ye et al.

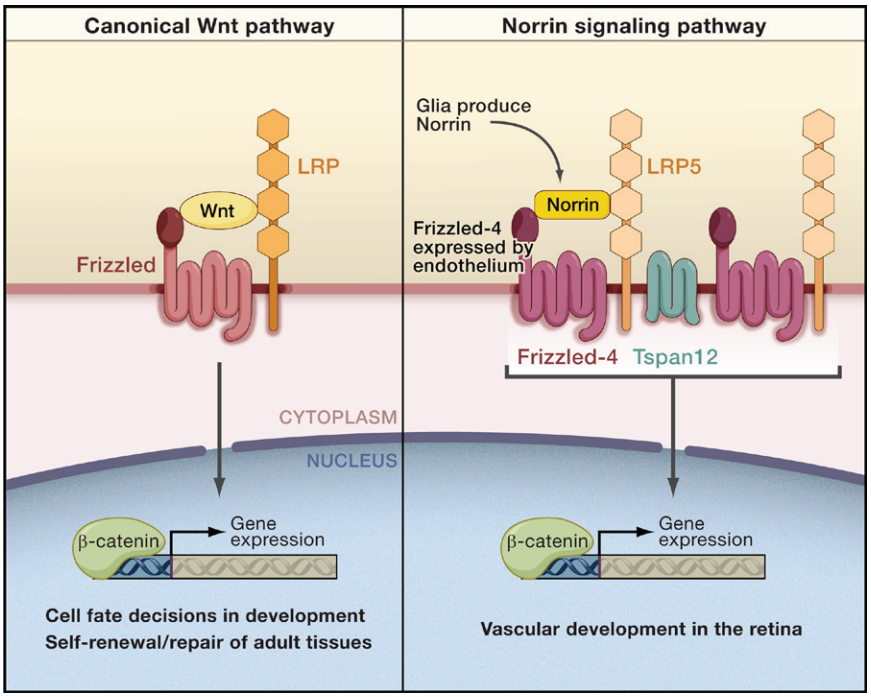

Figure 1. Norrin/β-Catenin Signaling in Theretina

(Left) In the canonical Wnt signaling pathway, Wnt ligand binds to any of 10 Frizzled receptors, which are associated with the coreceptors Lrp5 or Lrp6. Upon engagement with receptor, canonical Wnts such as Wnt3a activate downstream β-catenin, activating gene expression and resulting in regulation of a variety of processes in the embryo and the adult.

(Right) In the retina, the non-Wnt ligand Norrin is secreted by the glia and binds to the Wnt receptor Frizzled-4 (Frizzled-4) and its coreceptor Lrp5 on endothelial cells. Together with a newly identified component, the tetraspanin family member Tspan12, this complex initiates downstream β-catenin signaling, which results in vascularization of the retina during development. Mutations in Norrin, Frizzled-4, or Lrp5 (and potentially Tspan12) are associated with a variety of eye diseases characterized by poor vascularization of the retina (Junge et al., 2009; Ye et al., 2009).

show that the primary site of Frizzled-4 expression and signaling is in endothelial cells and that Muller glia cells are the principal source of Norrin (Figure 1). In vivo, loss of Frizzled-4 throughout the endothelium disrupts the cerebellar blood brain barrier, in agreement with an earlier report showing that Frizzled-4 mutant mice have progressive cerebellar degeneration (Wang et al., 2001). Meanwhile, generalized expression of Norrin signaling disrupts blood vessel formation (angiogenesis) in the mammalian embryo. Together, these findings indicate that the primary cellular defect in Norrie disease and related inherited retinopathies is vascular and support a broader role for Norrin/β-catenin signaling in angiogenesis. Microarray analysis pinpoints the endothelial marker gene *Sox17* as one of the early genes upregulated in response to Norrin-induced β-catenin signaling. Indeed, Sox17 overexpression rescues defects in capillary formation by cultured endothelial cells lacking Frizzled-4, implying that this transcription factor is a key mediator of the angiogenic program induced by Norrin signaling. Although vision is impaired in Frizzled-4 mutant mice due to retinal hypovascularization, the retina appears morphologically normal. Indeed, the authors perform electrophysiology experiments on Frizzled-4 mutant retinas ex vivo and show that chronic vascular insufficiency is compatible with long-term neuronal survival and the restoration of neurotransmission. Thus, the vision loss associated with these retinopathies may be reversible.

In a related study, Junge et al. identify a new member of the Norrin/Frizzled-4 receptor complex. The authors perform a reverse-genetics screen in mice involving 497 genes encoding transmembrane or secreted proteins. They find that mice carrying mutations in the tetraspanin family member Tspan12 phenocopy many of the defects observed in mice lacking Norrin, Frizzled-4, or Lrp5. In the eye, Tspan12 is uniquely expressed in the retinal vasculature, a pattern that is more restricted than that of Norrin, Frizzled-4, and Lrp5, consistent with the observations of Ye et al. Compound heterozygous mice lacking one allele each of *Tspan12* and *Norrin* or *Tspan12* and *Lrp5* show an increase in retinal pathology, demonstrating a genetic interaction between Tspan12 and two established components of the Norrin receptor-ligand complex.

Ye and colleagues go on to demonstrate that Tspan12 enhances Norrin/β-catenin signaling specifically. When overexpressed in cultured HEK293 cells, Tspan12 biochemically associates with the Norrin-receptor complex and significantly increases Norrin/β-catenin signaling but not Wnt/β-catenin signaling. And knockdown of Tspan12 expression abolishes transcriptional responses to Norrin but not Wnt3a in retinal endothelial cells. Importantly, none of a series of related tetraspanins can replace Tspan12 in these assays. Based on the observation that signaling defects caused by mutant forms of Norrin or Frizzled-4 are rescued by overexpression of Tspan12, the authors propose that Norrin and Tspan12 cooperatively promote multimerization of the Frizzled-4/Lrp5 complex to elicit β-catenin signaling (Figure 1). The phenotypic similarities of mice with mutations in Tspan12, Frizzled-4, Lrp5, or Norrin suggest that vascular retinopathies of undetermined genetic cause may result from mutations in *Tspan12*, a notion that will need to be tested.

The findings of Junge et al. demonstrate that non-Wnt ligands such as Norrin can activate β-catenin signaling through adaptation of the standard Frizzled/Lrp receptor configuration. A similar mechanism may be at play in another class of non-Wnt agonists of the β-catenin signaling pathway, the R-spondins; membrane-proximal events and receptor identity for the R-spondins are controversial.

Together, the findings of Ye et al. and Junge et al. advance our understanding of the spatial regulation of Norrin/β-catenin signaling in the retina. Given that Wnt/β-catenin signaling plays a central role in self-renewal and tissue regeneration in adult tissues, could Norrin/β-catenin signaling be involved in adult degenerative retinal vascular diseases such as diabetic retinopathy? Given that Norrin, Frizzled-4, Lrp5, and Tspan12 are still expressed in the postnatal retina, it may be possible to target the Norrin/β-catenin pathway to boost vascular regeneration in the eye. Such a strategy may open a new avenue for the treatment of the debilitating consequences of diseases like diabetes.

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Ferrying Wingless across the Synaptic Cleft

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Secreted Wnt morphogens mediate cell-cell communication, but the mechanism of Wnt transfer between cells is unknown. Korkut et al. (2009) report that the transmembrane protein Evi is a versatile carrier that guides Wingless to presynaptic terminals of motor neurons and then escorts it across the synaptic cleft. In postsynaptic muscles, Evi promotes Frizzled-2 trafficking.

Morphogens of the Wnt family provide critical positional information to cells during development (Logan and Nusse, 2004). In the nervous system Wnts play a role in axon pathfinding, dendritic development, and synaptogenesis, and defects in neuronal Wnt signaling are linked to neurodegenerative and cognitive disorders (Korkut and Budnik, 2009). Wnts are lipid-modified glycoproteins that activate a number of different pathways. Although we have a substantial understanding of the signaling cascades activated in Wnt-receiving cells, mechanisms controlling the secretion and subsequent dispersal of Wnt proteins have remained largely obscure. Three groups recently provided insight into the mechanism of Wnt trafficking through the identification of the conserved multipass transmembrane protein Evenness Interrupted/Wntless/Sprinter (Evi/Wls/Srt). Evi mediates bidirectional trafficking of Wnt-1/Wingless (Wg, the fly Wnt homolog) between the Golgi and the plasma membrane (Goodman et al., 2006 and reviewed in Ching and Nusse, 2006). In this issue of *Cell*, Vivian Budnik's group demonstrates that Evi is also involved in transporting Wnt to and across the synaptic cleft of the *Drosophila* neuromuscular junction, and in the trafficking of the Wnt receptor dFrizzled-2 in postsynaptic muscle cells.

Previous studies by this group showed that Wnt signaling is critical for neuromuscular development in *Drosophila*. In this system, activity-dependent Wg secretion from motor neuron terminals induces the Frizzled nuclear import pathway in the postsynaptic muscle cells (Korkut and Budnik, 2009; Figure 1). In this pathway, Wg signaling promotes the internalization, cleavage, and nuclear import of the Wg receptor dFrizzled-2 in Wg-receiving cells. The trafficking and nuclear import of dFrizzled-2 depends on a PDZ-domain protein called dGRIP.

Due to their hydrophobic nature, Wnts associate tightly with membranes and consequently do not diffuse in the extracellular milieu without assistance. How, then, does Wnt traverse the synaptic cleft of the neuromuscular junction? Previous studies proposed several different